PRODUCTION OF HYDROGEN PEROXIDE BY PHOTOSYSTEM II OF SPINACH CHLOROPLAST LAMELLAE

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1. Introduction

Isolated chloroplast lamellae produce the superoxide free radical ion (O_2^-) and hydrogen peroxide as a product of the dismutation of O_2^- [1–7]. This reaction is stimulated by autooxidizable electron acceptors of photosystem I and occurs in the presence of the natural electron acceptor system, ferredoxin and NADP, following the reduction of NADP [8,9]. The production of H_2O_2 by photosystem II in the presence of 15 μM dibromothymoquinone (DBMIB) was reported [10,11].

From the thermodynamic point of view, only the reducing site of photosystem I is negative enough (reviewed [12]) to function as the one-electron donor for oxygen (E_0^- for $O_2/O_2^{\cdot -}$ = –0.33 V [13]) if we assume that no divalent oxygen reduction occurs in chloroplasts [14].

A 2,3-dimethyl, 5,6-methylenedioxy *p*-benzoquinone-stimulated photophosphorylation coupled to oxygen uptake by isolated chloroplast lamellae was reported [15]. Dibromothymoquinone, an inhibitor of electron transport between the two photosystems [16,17] is not an inhibitor of this reaction [15].

We report here that the product of oxygen reduction by 2,3-dimethyl, 5,6-methylenedioxy *p*-benzoquinone is H_2O_2, apparently not derived via the dismutation of $O_2^{\cdot -}$ as in the case of the photosystem I-driven autooxidation of reduced low potential dyes [5–7].

Abbreviations: MV, methylviologen; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea

2. Materials and methods

Chloroplast lamellae were obtained from isolated intact spinach chloroplasts [19] by recentrifugation in a hypotonic buffer medium.

The products of oxygen photoreduction were determined after incubation of chloroplast lamellae, containing 100 μg chlorophyll, for 10 min at 18°C in Fernbach flasks (14 ml) with illumination (30 000 lux) from the bottom.

$O_2^{\cdot -}$ was determined as NO_2^-, produced from 0.5 mM hydroxylamine [9,20]. H_2O_2 was determined with the aid of NADH-peroxidase (Boehringer, Mannheim). Dibromothymoquinone (DBMIB) and 2,3-dimethyl, 5,6-methylenedioxy *p*-benzoquinone were gifts from Professor A. Trebst, Ruhr-Universität Bochum.

3. Results and discussion

Illuminated chloroplast lamellae in the absence of artificial electron acceptors produce H_2O_2 and, in the presence of 0.5 mM NH_2OH, nitrite. Nitrite formation from hydroxylamine can be used as indicator for O_2^- according to the equation:

$$NH_2OH + 2\ O_2^- + H^+ \longrightarrow NO_2^- + H_2O_2 + H_2O$$

[9,20,21]

3.1. *Effect of methylviologen (MV) on photosynthetic oxygen reduction*

Illumination of chloroplast lamellae in the presence of MV yields an increased production of both H_2O_2

Table 1
Effects of 2,3-dimethyl, 5,6-methylenedioxy *p*-benzoquinone (DIMEB), dibromothymoquinone (DBMIB) and methylviologen (MV) on H_2O_2 formation and hydroxylamine oxidation by illuminated chloroplast lamellae

Additions (10^{-5} M)	Activity (μmol/mg chlorophyll/h)			
	H_2O_2 formed		NO_2^- formed	
	− MV	+ MV	− MV	+ MV
None	11	36	4	13
DBMIB	9	15	0.2	0.3
DIMEB	30	35	2	0.6
DBMIB + DIMEB	25	33	0	0
DCMU	0	0	0	0
DCMU + DBMIB	1	1.2	0	0
DCMU + DIMEB	0.5	0.6	0	0
DCMU + DBMIB + DIMEB	2.5	3.6	0	0

The reaction system contained in 2 ml (mM): phosphate buffer (50), pH 7.8; NH_4Cl (2.5); $MgCl_2$ (2.5); chloroplast lamellae with 100 μg chlorophyll; MV (5×10^{-6} M) where indicated and, in the NO_2^- vessels, NH_2OH (0.5). The reactions were conducted for 10 min at 18°C in white light (30 000 lux)

and O_2^- (table 1) indicating monovalent oxygen reduction and dismutation of O_2^- [4–7].

3.2. *Influence of dibromothymoquinone and of 2,3-dimethyl, 5,6-methylenedioxy* p-*benzoquinone on photosynthetic oxygen reduction*

10^{-5} M dibromothymoquinone or 10^{-5} M 2,3-dimethyl, 5,6-methylenedioxy *p*-benzoquinone strongly inhibit monovalent oxygen reduction (NO_2^- formation from NH_2OH) by illuminated chloroplast lamellae, both in the presence and in the absence of methylviologen. H_2O_2 formation in the absence of methylviologen is stimulated by 10^{-5} M 2,3-dimethyl, 5,6-methylenedioxy *p*-benzoquinone and slightly inhibited by 10^{-5} M dibromothymoquinone (table 1). As shown in fig.1, stimulation of H_2O_2 (and inhibition of O_2^-) production (determined as NO_2^- formation from NH_2OH) strongly depends on the concentrations of either dibromothymoquinone or 2,3-dimethyl, 5,6-methylenedioxy *p*-benzoquinone. As compared to methylviologen (MV), low concentrations (10^{-6} M) of dibromothymoquinone (DBMIB) by approx. 50% inhibit both O_2^- (and H_2O_2 formation by illuminated chloroplast lamellae whereas higher concentrations (up to 10^{-3} M) stimulate H_2O_2) while decreasing O_2^- formation (fig.1a,b). 2,3-Dimethyl, 5,6-methylenedioxy *p*-benzoquinone (DIMEB) at 10^{-6} M is not an inhibitor of photosynthetic oxygen reduction. Increasing concentrations (up to 10^{-3} M) stimulate H_2O_2 formation (fig.1a) and similarly to dibromothymoquinone inhibit O_2^- formation (fig.1b).

DCMU, an inhibitor of photosynthetic electron transport blocks O_2^- formation in all the tested systems by 100% while approx. 10–20% of the original rate of H_2O_2 formation can still be observed in the presence of either dibromothymoquinone or of 2,3-dimethyl, 5,6-methylenedioxy *p*-benzoquinone, or a combination of both (table 1, [10]).

The above results are interpreted as follows: dibromothymoquinone as well as 2,3-dimethyl, 5,6-methylenedioxy *p*-benzoquinone are reduced by compound(s) located between the sites of inhibition by 10^{-6} M DCMU and by 10^{-6} M dibromothymoquinone (DBMIB) [18] and function as two-electron donors for oxygen forming H_2O_2 without O_2^- as intermediate. These reactions are different to the known photosystem I-driven oxygen reductions [4–7]. Whether this two-electron transport to some extent can bypass or accept electrons before the DCMU

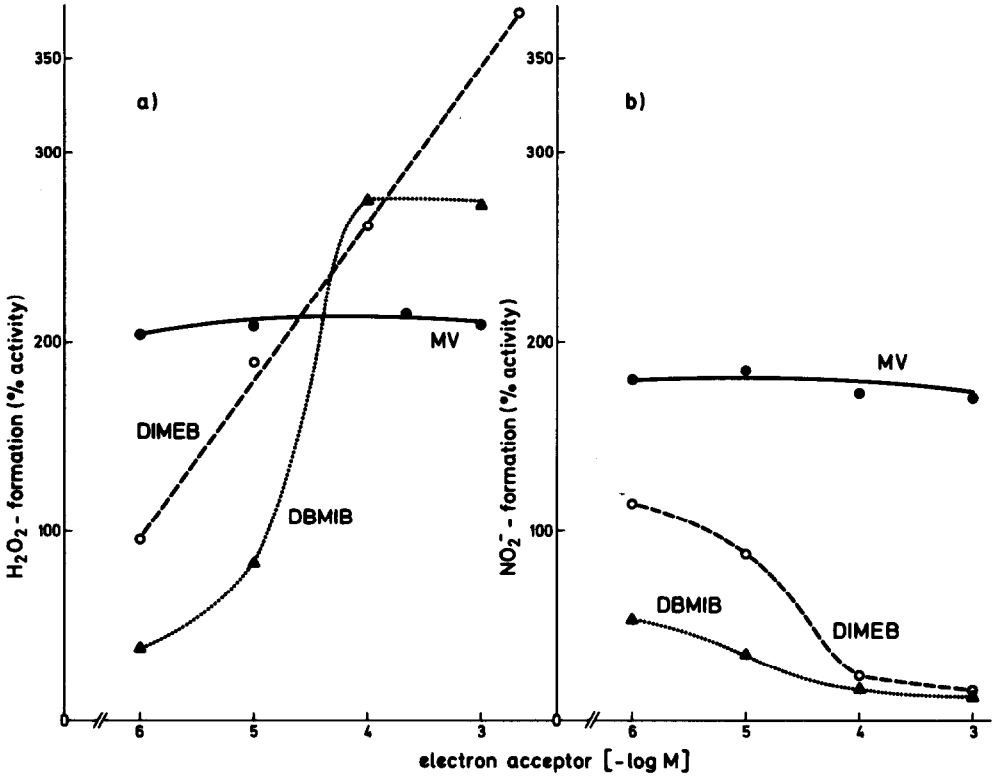

Fig.1. Effects of different concentrations of MV, 2,3-dimethyl, 5,6-methylenedioxy *p*-benzoquinone (DIMEB) and dibromothymoquinone (DBMIB) on H_2O_2 production (1a) and NH_2OH oxidation (1b) by illuminated chloroplast lamellae. The reaction conditions were as described in table 1. 100% activity corresponds to either 10 μmol H_2O_2 or to 2.5 μmol NO_2^- formed/mg chlorophyll/h in the absence of artificial electron acceptors.

block [10] needs further investigation. The question concerning the transition of one-electron to two-electron oxygen reduction mediated by compounds with different redox potentials is currently under investigation.

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